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A dualistic approach to investigate the remedial potential and edible property of *Pleurotus ostreatus* on hydrocarbon-contaminated soil

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ABSTRACT

Hydrocarbon soil contaminants are the major concern globally that causes an adverse impact on human health and reduces the functionality of ecosystem. Mycoremediation certainly has an edge over other bioremediation process in combating the targets most efficiently. This study attempts to investigate the potentials of an edible mushroom dualistically remediating the hydrocarbon-contaminated (HC) soil as well analyzing its impact on edibility. Many studies have been done over the past few decades, no attempts were made to analyze the presence or absence of toxicity sorption in edible organisms after remediation. HC soils from eight different petrol filling stations (Site A to H) of Coimbatore district were collected and analyzed for its physical and chemical properties. Based on physicochemical parameters, Site D1 that showed maximum total petroleum hydrocarbon (TPH) content (12,200 mg/kg) was chosen for this study. Five experimental sets were prepared by mixing the lignocellulose substance with HC soil at different ratio (Set I–Set V). Among the experimental sets, *P. ostreatus* showed maximum hydrocarbon degradation efficiency and well-defined fruiting body formation, high biological efficiency (140%), moisture (90%), and crude protein (32%) in Set III trials. Kinetic studies on TPH degradation fitted to the first-order kinetic model revealed a higher degradation rate constant, k (0.097 day-1) and lower biodegradation half-life t1/2 (7 days). Fourier transform infrared spectrum of *P. ostreatus* after remediation showed the complete absence of peak that corresponds to petroleum hydrocarbon, thereby preliminarily confirms the possibility of safe consumption.

1. INTRODUCTION

Petroleum spillage in the marine and terrestrial niches has a devastating effect on human community and other biota. Marine spills could not be completely eradicated unless it is drilled for oil or transported across the ocean, however, the release of petroleum hydrocarbons in these environments drastically alters the biological systems [1,2]. Compared to marine niches, the terrestrial ecosystem receives annually increasing amounts of oil spills. Contaminants in terrestrial sites need to be eradicated promptly as it leads to alter the pristine environment [3]. Petroleum compounds consist a mixture of aromatic and aliphatic hydrocarbons ranging from C4 to C12 carbon atoms [4]. Continuous exposure to hydrocarbons causes an adverse effect in human health such as irritations, respiratory problems, stress, nephrotoxicity, hemolytic anemia, and negative impacts on genetic and immune systems [5]. Besides the spillage, it results in limiting the nitrogen and phosphorous ratio in soil which causes detrimental effects in agriculture sectors [6]. Although many physical and chemical attempts (land filling, incineration, stabilization, and chemical oxidation) are employed for the removal of substantial portion of hydrocarbon spills, the remediation process was found to be unsatisfied as such practices lead to raise in atmospheric CO_2, NO_x, and So_x levels [7]. It was also reported that such implementation contaminates the ground water by producing hazardous leachates [8]. Moreover, physicochemical treatments have certain constrains such as expensive processing and requires involvement of more skilled persons [9]. Scientists are, therefore, intensively focusing on clean and economically feasible technology to mitigate the hydrocarbon contaminants for more than 2 decades [10,11].

Mycoremediation, employing fungi, has a peculiar role in degrading the toxic and xenobiotic compounds in the environment, since fungi due to its hyphal network can able to penetrate in to the soil matrix more efficiently than other organisms [12]. The extracellular enzymes secreted by fungal strains in the polluted environment have a cleavage effect on the polymeric substances, thereby insoluble compounds are hydrolyzed to soluble form which further absorbed and metabolized by the intracellular enzymes [13]. Mushrooms, macroscopic filamentous fungi, have the potential to degrade wide range of environmental pollutants, and convert them to useable products using different methods for instance biodegradation, biosorption, and bioconversion [14]. Consequently, such remediation strategies are considered to have an edge over other bioremediation process [15,16]. Mushroom secretes enzymes such as manganese-dependent peroxidases, lignin peroxidase,

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phenol oxidases, and H_2O_2-producing enzyme for the degradation of polycyclic aromatic hydrocarbons [17]. Like aromatic hydrocarbons, the major components persist in petroleum derivatives are aliphatic hydrocarbons (alkanes, branched alkanes, and cycloalkanes). Most of the fungal strikes over these toxic compounds are more resistant in particular for cyclic aliphatic hydrocarbons due to the absence of double bond in the terminal position [18]. Aliphatic hydrocarbon degradation does not rely on the same enzymatic complex which is used to degrade the aromatic compounds in mushrooms. Instead, it requires a heme protein, cytochrome P450 monooxygenases that involve a hydroxylation reaction where an oxygen atom is introduced in the terminal methyl group to form alcohol and then to corresponding aldehyde and fatty acid [19]. The resultant fatty acid subsequently undergoes p-oxidation to form acyl-CoA ester and eventually catabolized in the TCA cycle [20].

Mushrooms, apart from its versatile role in remediating the contaminants, famed for its rich source of protein, fiber, and antioxidants. A special consideration and extensive research should be exercised in consuming edible mushroom cultivated in severe environmental contaminated places [21]. Meager reports were discussed to ensure the safety consumption of edible mushrooms after they were exploited for remediation purpose [22]. Therefore, the present investigation dualistically focused to degrade the petroleum hydrocarbon-contaminated (HC) soil using an edible oyster mushroom (*Pleurotus ostreatus)* and subsequently attempts to study the characteristic changes for any hindrance or toxicity in the edible properties of mushroom.

2. MATERIALS AND METHODS

2.1. Collection of Soil Samples

Soil samples were obtained in eight different fuel filling sites of Coimbatore District (Site-A: Ukkadam; B: Pothanur; C: Malumachampatti, D: Kinathukadavu; E: Vadachitur; F: Negamam; G: Thoppampatti, and H: Pollachi), Tamil Nadu, India. Samples were taken from two points of each fuel stations, one at the top layer (Site A0, B0, C0, D0, E0, F0, G0, and H0) and second at 1 m depth from the ground surface (Site A1, B1, C1, D1, E1, F1, G1, and H1). Samples were sieved using 2 mm mesh to obtain a homogenous mixture and were transported immediately to laboratory for the physicochemical analysis.

2.2. Physicochemical Analysis of HC Soil

HC soil was analyzed for the soil texture, porosity [23], permeability [24], and moisture content (gravimetric method) [25]. The total nitrogen [26] content, phosphorous [27,28], and total carbon content were determined according to the standard procedure (Shimadzu-TOC-V_{CSH}, Japan) [29].

The collected soil samples were homogenized and precisely 2 g of soil was weighed in 100 mL container. One-fourth strength of Ringer's solution (20 mL) was added to the content and vortexed for 2 min. After vortexing, the samples were sonicated using sonicator (Thermo Fisher Scientific, USA) and subsequently allowed to stand for 2 min. An aliquot of 10 mL of soil suspension was removed and serially diluted (10^{-5}–10^{-4}) in the aforementioned strength of Ringer's solution. To determine the indigenous microbes, 10 mL of each dilution was added to the $^1/4$ strength of LB medium for bacteria using pour plating technique and acidified potato dextrose agar containing streptomycin (1 mg/100 ml) for fungal enumeration [30]. The samples were incubated for 24 h at 30°C (bacteria) while fungi were incubated at room temperature (3–5 days), further, the colony-forming units were enumerated and expressed as CFU g^{-1} of dry soil.

2.3. Total Petroleum Hydrocarbon (TPH) Estimation

TPH content of the soil was examined by cold toluene extraction method [31]. Precooled 20 mL of toluene was added to the flask containing 10 g of petrol contaminated soil sample. The flask was placed in the orbital shaker for 30 min. The liquid phase was collected by filtration and the extract was measured at 420 nm in the ultraviolet (UV) spectrophotometer (UV–Vis Spec. Elico SL159). Soil TPH was determined using a standard curve prepared with toluene diluted oil. The percentage of degradation (D) was calculated as

D = TPHi - TPHr/TPHi

Where, TPHi and TPHr correspond to the initial and residual TPH concentration, respectively.

2.4. Kinetic Analysis

TPH degradation data were fitted with a first-order kinetic model. The reaction kinetics is defined by the equation

$$C = C_0 e^{-kt} \quad (1)$$

Where, C is the hydrocarbon content at the time t and C0 is the initial hydrocarbon concentration (mg kg^{-1}), k is the degradation rate constant (day^{-1}) [32]. Eq. (1) was represented in linear integrated form

$$Ln (C/C_0) = -ktq \quad (2)$$

The degradation rate constant (k) was determined using Eq. (2). The half-life time (t ½) of the remedial activity was calculated using Eq. (3) [33,34].

$$t½ = ln (2)/k \quad (3)$$

Where, k is the rate constant determined from Eq. (2).

2.5. Substrate and Spawn Processing

Paddy straw was collected from the local farm near Pollachi, Tamil Nadu. The paddy straw was approximately chopped to 2 cm and soaked in water for overnight and air-dried to remove excess moisture content. HC soil used in the study was shed into the perforated sterile polyethylene bags mixed with paddy straw at five different ratios (Set I – Sole Paddy straw; Set II – Paddy straw: HC soil [1:1]; Set III – Paddy straw: HC soil [2:1]; Set IV – Paddy straw: HC soil [1:2]; and Set V – Sole HC soil). Pure culture of *P. ostreatus* was obtained from Tamil Nadu Agricultural University, Coimbatore (India). The collected spawn was weighed equally (5 g) and added to all the five experimental sets. All the tested sets were performed in triplicates under appropriate conditions (temperature – 22–25°C; humidity – 60%) and the mushroom growth was examined after a period of 21 days.

2.6. Determination of Yield and Biological Efficiency (BE)

The yield of the mushroom was calculated by weighing the fruiting body and percentage of BE was determined [35].

BE (%) = (fresh weight of mushroom ×100)/substrate dry weight

2.7. Estimation of Crude Protein and Moisture

Crude protein was estimated by multiplying the factor 6.25 with total Kjeldahl nitrogen obtained (Kel Plus Kes 6L). To determine the

moisture content, 1 g of fresh mushroom was weighed and kept at 60°C overnight until for obtaining a standard weight. The moisture content of mushroom was estimated [36].

Moisture content (%) = 100 × (W1 - W2)/W1

Where, W1= weight of fresh sample and W2 = weight of dry sample.

2.8. Fourier transform Infrared (FTIR) Analysis

P. ostreatus from all the tested sets were collected and washed in distilled water to remove the debris. The mushroom samples were dissected manually and hot dried in the oven at 60°C to constant weight. Dried samples were pulverized using grinder and sieved with 80 µm mesh [37]. About 1 mg of sample was mixed with 100 mg of spectroscopic grade potassium bromide to prepare a translucent sample disc. The FTIR spectrum was analyzed between 400 and 4000 cm^{-1} range. Each spectrum was performed with 40 scans min^{-1} (Shimadzu, IR Affinity 1, and Japan).

3. RESULTS AND DISCUSSION

3.1. HC Soil Analysis

The HC soil samples collected from eight different sites [Figure 1] were characterized for the physicochemical parameters [Table 1]. The texture of the soil was observed to be red loamy (Site A_0 to Site D1) and sandy loamy (Site E_0 to Site H1). The soil pH was found to be near neutral to alkaline (6.9–8.4). The total nitrogen content was noted to be 0.12–0.22%, carbon content was in the range of 0.58–1.66%, and phosphorous was found to be 10.2–33.89 mg/kg^{-1}. Soil porosity was noted to be 15–31% and permeability was found to be in the range of 0.032–0.065 m/h. Successful hydrocarbon degradation is based on soil characteristics precisely due to certain significant factors that hinder the remedial effect such as soil texture, permeability, pH, water holding capacity, nutrients, oxygen, and microbial population [38].

The indigenous microbial count in the HC soils from A_0 to H1 sites showed distinct results. In the present study, the bacterial count in the red loamy regions was observed to be high (4.96×10^6–5.82×10^6 cfu g^{-1}) whereas in sandy loam, it showed a reduced count (2.79×10^6–3.40×10^6 cfu g^{-1}). Likewise, the fungal enumeration was observed to be maximum in red loam (0.13×10^5–0.23×10^5) regions and minimum in sandy loams (0.06×10^5–0.11 m $\times 10^5$). Microbial distribution in different soil textures is based on the nutrient content and soil moisture in the environment [39]. The results noticed from our study also proved that unlike fine textured soil (red loam, Site A to D1), the course textured soil (sandy loam, Site E to H1) did not influence the microbial count. This might be due to the slight alkaline pH as well as the availability of organic matter and nitrogen content that persist in red loamy regions.

3.2. TPH Analysis

TPH content was analyzed in all the selected sites (Site-A to Site-H) [Figure 2]. The graph showed maximum TPH content was noticed in

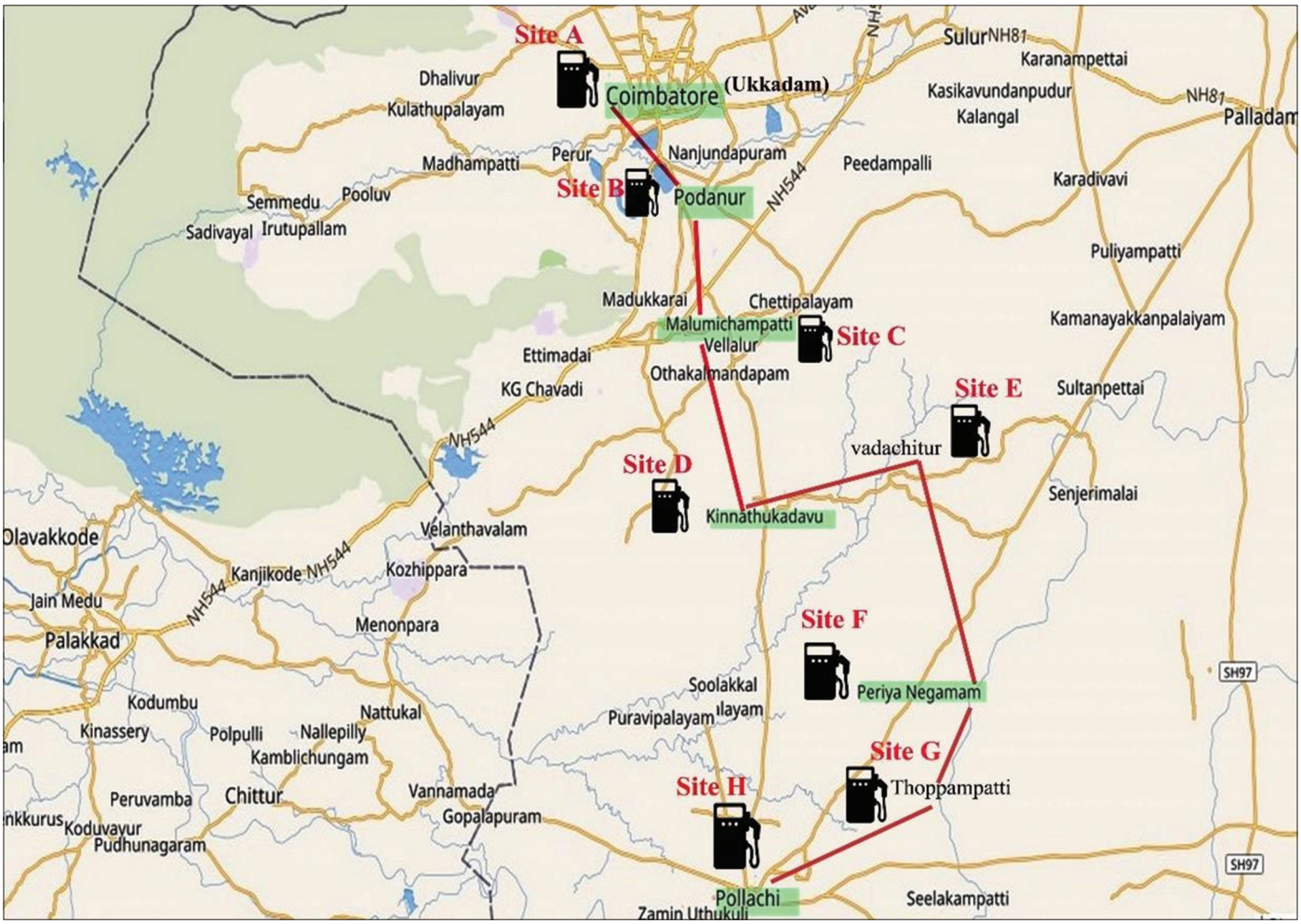

Figure 1: Map showing the sample collected sites near petrol filing points

Table 1: Physicochemical properties of the soil at collected hydrocarbon-contaminated sites

Parameters	Units	Hydrocarbon-contaminated sites																			
		A_0	A_1	B_0	B_1	C_0	C_1	D_0	D_1	E_0	E_1	F_0	F_1	G_0	G_1	H_0	H_1
Type		Red loam	Red loam	Red loamy	Red loamy	Red loam	Red loam	Red loam	Red loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sandy loam
Texture		Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse	Coarse
Moisture	%	7.0	7.5	8.2	8.9	6.5	7.0	7.2	7.1	6.8	7.3	5.3	5.7	6.2	6.8	4.2	4.9
Porosity	%	31	27	26	22	19	17	16	15	29	27	30	28	22	20	23	20
Permeability	m/h	0.065	0.060	0.055	0.047	0.040	0.038	0.034	0.032	0.062	0.058	0.064	0.060	0.047	0.043	0.049	0.043
Total N2	%	0.20	0.22	0.19	0.20	0.16	0.14	0.16	0.13	0.21	0.20	0.17	0.16	0.13	0.12	0.16	0.15
Total C	%	0.58	0.69	0.78	0.84	1.25	1.36	1.26.	1.66	0.86	0.82	0.97	1.05	0.77	0.82	0.96	1.15
Phosphorous	mg/k-1	12.4	10.2	10.89	13.6	32.5	33.89	20.7	23.6	32.7	20.7	24.2	28.6	21.6	19.5	18.2	17
pH	-	7.8	7.6	7.9	7.7	7.1	6.9	8.1	7.6	8.4	8.1	7.2	7.1	7.8	7.6	8.1	7.9
Bacterial	Cfu/g	5.52×10^6	5.82×10^6	5.22×10^6	5.42×10^6	5.67×10^6	5.73×10^6	4.96×10^6	4.98×10^6	3.33×10^6	3.12×10^6	2.89×10^6	2.79×10^6	3.12×10^6	3.26×10^6	3.40×10^6	3.29×10^6
Fungi	Cfu/g	0.22×10^5	0.23×10^5	0.19×10^5	0.24×10^5	0.15×10^5	0.14×10^5	0.13×10^5	0.14×10^5	0.09×10^5	0.08×10^5	0.07×10^5	0.06×10^5	0.10×10^5	0.09×10^5	0.10×10^5	0.11×10^5

all the soils that were collected at 1 m depth compared to surface soils. Among the soils collected at depth, Site A1 and Site D1 exhibited high hydrocarbon contaminants of 11,550 mg/kg and 12,200 mg/kg, respectively. The variation of TPH content in different sites may also be due to the activity of extracellular enzymes secreted either by native fungal strains or it depends on the availability of hydrocarbon utilizing bacteria in the contaminated sites [40]. Microbes that persist in the HC soil have a positive effect on the controlling TPH content both in the liquid and solid phases as these organisms are very well acclimated to the environmental conditions [41]. In our previous study, we identified indigenous microbes (hydrocarbon utilizing bacteria) present in HC sites that have a synergetic effect on the rate of hydrocarbon degradation (data not shown) [42].

Site A1 and Site D1, besides its high TPH content, also consist of increased microbial count. Interestingly, it has been noticed a distinct features between these two sites where Site D1 has a low soil porosity 15% and permeability 0.032 m/h when compared to Site A1 (27% soil porosity and 0.060 m/h soil permeability). The observation in this study clearly reveals that the Site D1 that possesses low soil porosity and lesser permeability has the ability to clench hydrocarbons in the soil for years regardless to enriched microbial population.

3.3. Mycoremediation Study

Among the HC sites, Site D1 was chosen for mycoremediation study based in the earlier findings. HC soil from Site D1 was added to paddy straw amended with the spawn of edible oyster mushroom (*P. ostreatus).* The experiment was performed with five different experimental sets to observe the degradation efficacy of hydrocarbon as well the impact of mushroom growth. Figure 3 shows the distinct development of fruiting body in different sets of paddy straw: HC soil after a period of 21 days. The reason for the difference in the yield of mushroom may be due to the availability of nutrients in each experimental set. Our results agree with the findings of Naraian *et al.,* where the growth of the mushroom and primordial development is based on the lignocellulosic substance, particularly on the C/N ratio [43]. Subsequent findings in our study also showed maximum BE (140.26%) and the highest crude protein content (32%) was recorded in Set III (paddy straw: HC soil [2:1]) when compared to other tested sets. Nearly similar (BE%) was also observed in Set I and Set II [Figure 4]. Moisture in all the tested sets was found to have no significant difference despite increased paddy straw concentration has a slight impact on its content (i.e., Set I – 87% and Set III – 90%). The reason may be due to suitable nutrient ratio increases the yield and the fruiting body efficacy available in Set III. Similar type of results has been stated by Yang *et al.* that the increased mycelia growth and fruiting bodies depend on the optimal condition of the carbon and nitrogen ratio [44].

3.4. Biomass Yield and Weight

Figure 5 shows the TPH degradation rate of the soils in all the experimental sets (except Set I) which showed interesting patterns. Simultaneously estimation for highest mushroom yield (1402 g/kg of substrate) by *P. ostreatus* was observed in Set III (paddy straw: HC soil [2:1]). The TPH degradation rate in Set III was 87% which was the maximum among the tested sets. The mushroom yield both in Set I (sole paddy straw) and Set II (paddy straw: HC soil [1:1]) showed similar pattern of productivity (around 1200 g/kg of substrate) followed by the Set IV (1054 g/kg of substrate). Degradation rate of 64% and 42% was achieved in the Set II and Set IV, respectively. The interesting fact observed in our study was the yield of the mushroom in Set V (sole HC soil) did not show a remarkable growth, however, 452 g/kg of substrate

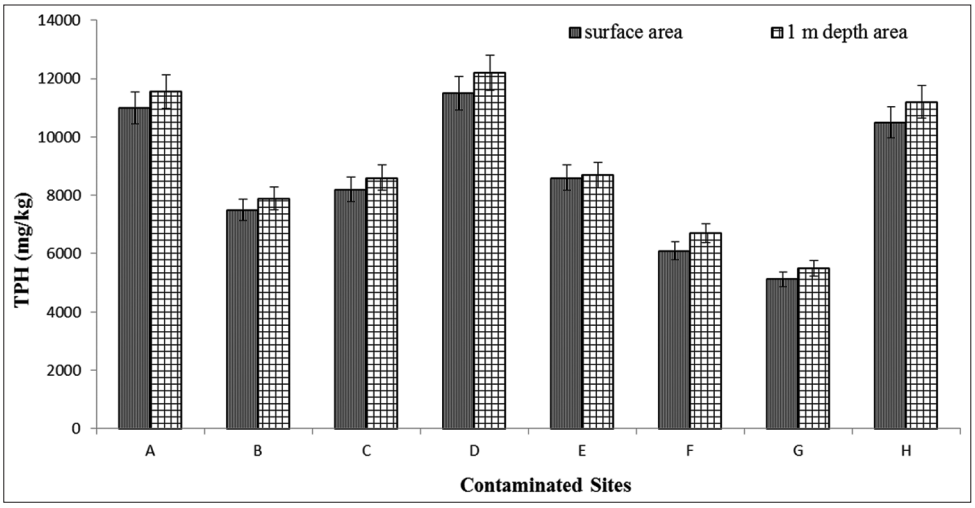

Figure 2: Total petroleum hydrocarbon content in different hydrocarbon-contaminated sites. Data are the mean ± standard deviation of three replicates

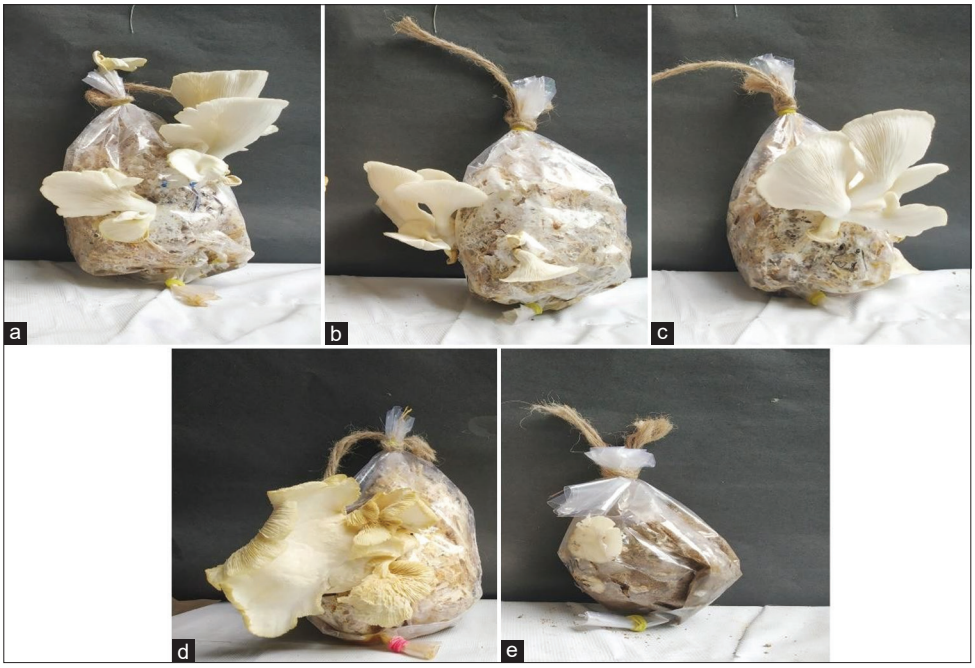

Figure 3: Fruiting body formation of *Pleurotus ostreatus* in different experimental sets after 21 days. (a) Set I – Sole paddy straw; (b) Set II – paddy straw: Hydrocarbon-contaminated (HC) soil (1:1); (c) Set III – paddy straw: HC soil (2:1); (d) Set IV – paddy straw: HC soil (1:2); and (e) Set V – sole HC soil

of yield was noticed with 23% of degradation efficiency. These results obviously showed that mushroom growth and remedial effect solely depend on the appropriate content of C: N sources available in the experimental sets. In the findings of Leys *et al.,* low supplement of carbon and nitrogen source did not support the growth of the organism and hence observed a lower degradation rate [45]. Supporting to our study, Teng *et al.* showed carbon and nitrogen when amended with 10:1 ratio significantly elicited the hydrocarbon degradation rate when compared to the C: N either with 25:1 or 40:1 [46]. Besides growth, in our observation, *P. ostreatus* have capacity to degrade the hydrocarbon pollutant possibly by secreting extracellular enzymes such as hydroxylases and monooxygenases. Our findings have a correlation with the reports of Bogan and Lamar [47] where the degradation of petroleum HC soil is mediated by the extracellular enzymes, which is also reported by Ekundayo [48].

3.5. Kinetic Analysis

Kinetic analysis for studying the degradation rate of HC soils by *P. ostreatus* in various treatments was fitted well to first-order kinetics [Table 2]. Highest degradation rate constant was observed in the HC soil of Set III (0.097 day^{-1}). Subsequently, Set II, Set IV, and Set V HC soil showed the degradation rate constant (k) as 0.048 day^{-1}, 0.025 day^{-1}, and 0.012 day^{-1}, respectively. The biological half-life ($t_{½}$) values of various treatments on hydrocarbon degradation observed in Set II, Set III, Set IV, and Set V are 14, 7, 28, and 58 days, respectively. Experimental Set III (paddy straw: HC soil [2:1]) that showed highest biodegradation rate constant has lowest half-life time when compared to other sets. Thus, the data representing in kinetic model analysis can conclude that the rate constant (k) has a significant correlation with TPH degradation rate (%). Higher biodegradation rate is directly proportional to higher degradation rate constant and lower half-life

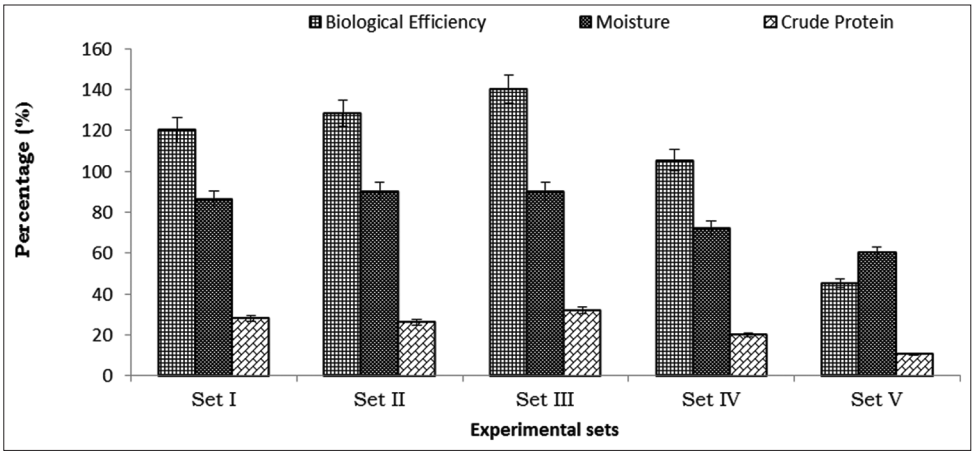

Figure 4: Effect of different experimental sets on biological efficiency, moisture, and protein of *Pleurotus ostreatus* (Bars = Standard Deviation)

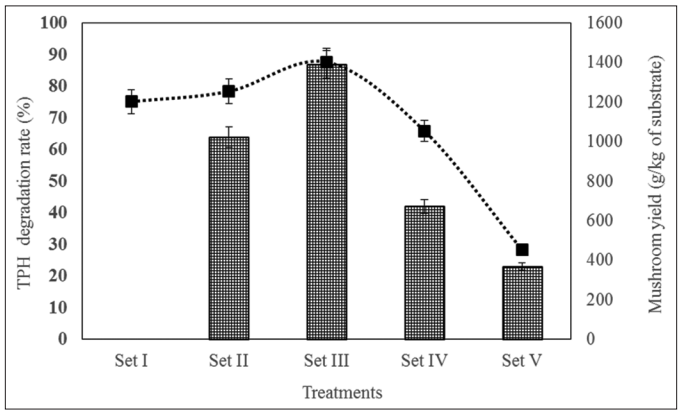

Figure 5: Total petroleum hydrocarbon (TPH) degradation rate and biomass content of *Pleurotus ostreatus* after 21 days. The bars represent the TPH degradation (%) and the dotted lines correspond to the yield of mushroom (g/kg^{-1})

Table 2: Biodegradation rate constant (k) and half-life (t½) time in various treatments of hydrocarbon-contaminated soil

Treatments	Rate constant (k^{-1} day)	Half-life time (t½)
Set I (Sole paddy straw)	n/a	n/a
Set II (Paddy straw: HC soil [1:1])	0.048	14
Set III (Paddy straw: HC soil [2:1])	0.097	7
Set IV (Paddy straw: HC soil [1:2])	0.025	28
Set V (sole HC soil)	0.012	58

time [34]. Both experimental study and kinetic analysis showed that the perceived progressed remedial effect noticed by *P. ostreatus* in Set III may be attributed to the better growth due to the suitable C/N sources, thereby increases secretion of oxidative extracellular enzymes that catalyze an effective mycoremediation process.

3.6. FTIR Analysis

The harvested oyster mushroom grown in the different sets was analyzed for the functional group changes by FTIR spectroscopy [Figure 6]. Besides the remediation potentials, *P. ostreatus* is a highly nutritious diet where it is mainly considered as a source of single cell protein. Therefore, in our study, we focused on analyzing the characteristic changes in *P. ostreatus* particularly so that the hydrocarbon contaminants do not affect the nature of edibility either by uptaking the toxic compound or by limiting the nutritional property in the mushroom. Mostly FTIR analysis in bioremedial studies so far was unidirectional, that is, focusing primarily on pollutant degradation, still, it is also essential to study the fate of contaminants and its impact on biological systems. In our findings, FTIR spectra of *P. ostreatus* cultivated in Set I and Set III have mostly similar banding assignments [Table 3].

The broad peak near 3400 cm^{-1} represents the presence of O-H stretch in the hydrogen bonds. The strong peak near 2930 cm^{-1} corresponds to C-H stretch which confirms the presence of pyranose ring (glucan or chitosan). Bands near 1630 and 1516.05 cm^{-1} attribute to C = O stretch (Amide I) and N-H (Amide II), indicate the presence of proteins. The latter band is observed in Set I and the former is observed in both Set I and Set III. The characteristic bands near 1076.28 and 887.26 cm^{-1} confirm the presence fingerprint of carbohydrate such as mannogalacton and β-glucans. FTIR spectra of *P. ostreatus* cultivated in Set IV and Set V, reveled distinct banding assignments when compared to earlier two experimental sets. The broad O-H stretch near 3400 cm^{-1} was completely limited in Set IV and Set V, however, some vibration has been noticed. Similarly, the spectra of mushroom from both the sets showed vibration in protein peaks (Amide I and Amide II) near 1600 and 1500 cm^{-1}. Nevertheless, in Set IV and Set V, sharp bands have been noticed in the carbohydrate fingerprint regions (1200–1000 cm^{-1} and 900–700 cm^{-1}). In our study, mushroom grown in all the tested experimental sets showed a notable carbohydrate characteristic peak (regions, 1200–1000 cm^{-1}) in the FTIR analysis and strikingly because of the carbohydrate (polysaccharide, particularly glucan) content, the difference in the yield of the mushroom has been observed. The structure and weight of the edible *P. ostreatus* are subjected to the presence of β-glucans in additional to its bioactive property [49]. This finding is also supported by Synytsya *et al.,* where the glucan content is directly proportional to the fruiting body [50]. Characteristic strong bands near 1600 cm^{-1} (1631.78 cm^{-1} in Set I; 1635.64 cm^{-1} in Set III) attributed to Amide I bands (C=O stretch) which represent the presence of peptide bond and similar type of observation was also reported [51]. The strong Amide I bands observed by FTIR in our study influence the protein content in *P. ostreatus.* This result has a significant correlation with our earlier analysis where Set III among all other sets showed maximum protein content. This may

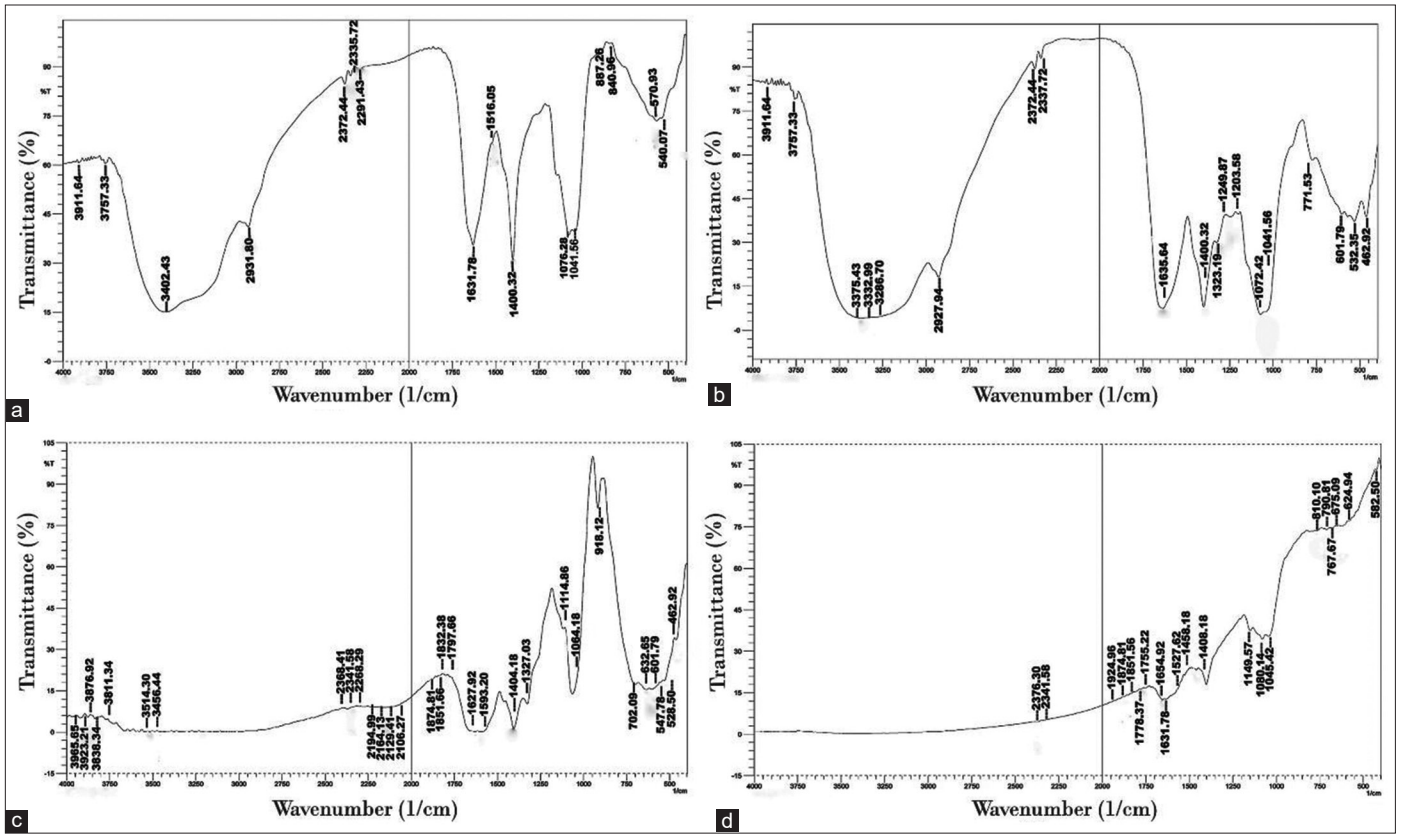

Figure 6: Characteristic bands of Fourier transform infrared spectrum of *Pleurotus ostreatus* grown in different experimental sets. (a) Set I – sole paddy straw; (b) Set III – paddy straw: Hydrocarbon-contaminated (HC) soil (2:1); (c) Set IV – paddy straw: HC soil (1:2); and (d) Set V – sole HC soil

Table 3: The assigned bands (cm^{-1}) obtained from the FTIR spectra of *Pleurotus ostreatus* grown in different experimental sets. Bands given in parentheses correspond to the standard bands of edible oyster mushroom

Set I (sole paddy straw)	Set III (paddy straw: HC soil [2:1])	Set IV (paddy straw: HC soil [1:2])	Set V (sole HC soil)
3911.64–3402.43 (br, O-H str, chitin)	3911.64–3286.70 (br, O-Hstr, chitin)	3965.65–3213.41 (vbr, O-H str, Chitin)	-
2931.80 (vs, C-H str pyranose ring; glucan/chitosan)	2927.94 (vs, C-H str, pyranose ring; glucan/chitosan)	-	-
2372.44–2291.43 (sh, C-H str)	2372.44–2086.98 (sh, C-H str)	2368.59–2106.27 (vbr, C-H str)	2376.30–2341.58 (vbr, C-H str)
-	-	1874.81–1797.66 (vbr, Double bond)	1924.96–1755.22 (vbr, Double bond)
1631.78 (vs, Amide I, C=O stretching of the peptide bond)	1635.64 (vs, Amide I, C=O stretching of the peptide bond)	1627.92 (vbr, Amide I, C=O stretching of the peptide bond)	1654.92,1631.78 (vbr, Amide I, C=O stretching of the peptide bond)
1516.05 (Amide II band; glucan)	-	1593.20 (br, Amide II band; glucan)	1527.62 (vbr, Amide II band; glucan)
1400.32 (vs, C=O Str)	1400.32 (vs, C=O Str)	1404.18 (sh, C=O Str)	1458.18–1404.18 (sh, C=O Str)
-	1323.17 (sh, O-H bending, glucan)	1327.03 (sh, O-H bending, glucan)	-
-	1249.87–1203.58 (vbr, carbohydrate fingerprint zone)	-	-
1076.28, 1041.56 (vs, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1072.42, 1041.56 (vs, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1064.71 (sh, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)	1149.57–1045.42 (vbr, carbohydrate fingerprint zone; confirms presence of chitin or chitosan)
887.26, 840.96 (sh, confirmatory band for Mannan type glucan)	771.53 (sh, confirmatory band for Mannan type glucan)	918.12 (sh, confirmatory band for Mannan type glucan)	810.10-767 (vbr, confirmatory band for Mannan type glucan)

Vbr: Vibration; br: Broad, sh: Sharp, vs: Very strong

be due to the availability of suitable C/N ratio, namely, paddy straw: HC soil (2:1) that influences the protein substance in the mushroom. The difference in nutrient content of the mushroom has been noticed with respect to the substrates [52,53]. Biosorption is

an efficient process for treating the environmental pollutants either live or dead state [54]. Few literatures have discussed on biosorption properties of mushroom on toxic substances when grown in polluted environments. In contrast, from our study, FTIR spectra of *P. ostreatus* analyzed in all the experimental sets interestingly have showed complete absence of aliphatic and aromatic stretches (band assignment, 3150 cm^{-1}–2800 cm^{-1}), that is, the banding assignments particularly for petroleum hydrocarbons. Similar type of microbial activity was significantly noticed with few research findings [55,56]. Eventually from our study, it is evident that the edible mushroom utilized for bioremediation has no biosorption of toxic compounds. The functional groups of *P. ostreatus* cultivated in Set III and Set I are similar, thus, it clearly shows that this oyster mushroom beyond its potential remedial effect can be consumed safely since the edible property is not either altered or reduced considerably.

4. CONCLUSION

Mycoremediation ensures the possibility of developing a clean technology. Inadequate knowledge was available so far on directly augmenting mushrooms in crude HC soil. The present study concludes adding paddy straw with raw HC soil in 2:1 ratio stimulates the remedial activity of *P. ostreatus* against hydrocarbons over a short period of time. The current spectral study proved the complete absence of hydrocarbon peaks in the remediated mushroom through spectroscopic analysis which can be considered as an interesting fact for safe edibility. However, a clear knowledge on the fate of hydrocarbon degradation in this context has to be understood in terms of mushroom enzymology in future.

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6. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

7. ETHICAL APPROVALS

Ethical approval was not required for this study.

8. DATA AVAILABILITY

The data set generated during the current study is available from the corresponding author on reasonable request.

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